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Prenylated flavonoids of *Erythrina lysistemon* grown in Egypt

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Abstract

Three prenylated flavonoid derivatives; 5,7,4′-trihydroxy-8-(3‴-methylbut-2‴-enyl)-6-(2″-hydroxy-3″-methylbut-3″enyl) isoflavone (isoerysenegalensein E), 5,7,2′-trihydroxy-4′-methoxy-5′-(3″-methylbut-2″-enyl) isoflavanone (lysisteisoflavanone), 5, 4′-dihydroxy-6-(3‴-methylbut-2‴-enyl)-2″-hydroxyisopropyl dihydrofurano [4″,5″:8,7] isoflavone (isosenegalensin), together with the four known flavonoids abyssinone V-4′-methylether, alpinumisoflavone, wighteone and burttinone were isolated from the stem bark of *Erythrina lysistemon* Hutch. (Leguminosae). Structures were elucidated by spectroscopic methods.

Keywords: *Erythrina lysistemon*; Leguminosae; Prenylated flavonoids; Isoerysenegalensein E; Lysisteisoflavanone; Isosenegalensin

1. Introduction

The genus *Erythrina* (Leguminosae) includes numerous species distributed in tropical and subtropical regions of the world (Bever, 1986). These plants have a significant history of medicinal use for the treatment of diseases such as female infertility, stomach pain and gonorrhea (Irvine, 1961) and they are well known for their alkaloid and flavonoid content. In the past two decades, more than 50 flavonoids have been isolated from *Erythrina spp*, the majority of them are prenylated (Saleh, 1992). *Erythrina* alkaloids have a curare-like action (Dyke and Quessy, 1981). Prenylated flavonoids have been found to display a variety of biological activities such as behavioral depression and muscle relaxation; they are also known to be antihypertensive and to have β_1-adrenergic inhibition and antimicrobial activities (Fomum et al., 1986).

Erythrina lysistemon is one of two *Erythrina* species cultivated in Egypt as an ornamental plant. In a previous study three novel glycodienoid alkaloids were isolated from this species (Amer et al., 1991). The present study was carried out aiming to isolate the flavonoidal components of the plant and test them biologically.

2. Results and discussion

The UV data (see Experimental) of compound **3** showed maximum absorption at 272 nm together with the ^{1}H NMR (Table 1) singlet at δ 7.88 ppm correlated to a ^{13}C NMR (Table 1) signal at 152.8 ppm by an HMQC experiment suggesting the presence of an isoflavone skeleton (Mabry et al., 1970; Agrawal, 1989). EI-MS (see experimental) showed [M^+] at m/z 422 in complete agreement with the suggested formula $C_{25}H_{26}O_6$. In the ^{1}H NMR spectrum the two doublets at δ 6.82 and δ 7.32 and the mass fragment in the MS spectrum at m/z 118 indicated the presence of a hydroxyl group at C-4′ of a monosubstituted ring B. On the other hand, the absence of other aromatic proton signals indicated that ring A was fully substituted. The 12 nm bathochromic shift observed in the NaOAc spectrum, the inability of $AlCl_3$ to produce any bathochromic shift (Tsukayama et al., 1992), together with the ^{13}C NMR chemical shifts (160.8, 157.9 and 109.2 ppm) (Table 1) suggested the presence of free hydroxyls at C-5, C-7 and an angular substituent at C-6. The ^{1}H NMR signals at δ 3.47, 5.22, 1.81 and 1.69 with their corresponding carbons were assigned for a prenyl group

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Table 1
Spectral data for compounds[a] **2**, **3**, **6** and **8**

Position	**2**[b]	**3**[c]			**6**[c]		**8**[c]		
	^{13}C	^{13}C	^{1}H	HMBC	^{13}C	^{1}H	^{13}C	^{1}H	HMBC
2	152.5	152.8	7.88 (H, *s*)	C-3, C-4, C-9	69.6	4.61 (1H, *bd*, *J* = 7.6 Hz) 4.76 (1H, *m*)	152.7	7.88 (1H, *s*)	C-3, C-4, C-9
3	123.5	123.1			45.3	3.98 (1H, *m*)	123.2		
4	180.9	181.4			197.2		181.4		
5	157	157.9			164.8		156.2		
6	105.8	109.2			96.9	5.92 (2H, *s*)	102.2		
7	159.3	160.8			166.3		164.1		
8	94.8	107.3			95.5	5.92 (2H, *s*)	108.6		
9	157.1	154			163.3		154.9		
10	105.3	105.5			101.8		106.7		
1′	121.6	122.2			122.9		122.7		
2′	130	130.3	7.32 (2H, *d*, *J* = 8 Hz)	C-1′, C-4′,	153.8		130.2	7.31 (2H, *d*, *J* = 7.8 Hz)	C-3, C-4′
3′	115.3	115.7	6.82 (2H, *d*, *J* = 8 Hz)	C-1′	100.7	6.43 (1H, *s*)	115.6	6.80 (2H, *d*, *J* = 7.8 Hz)	C-3, C-4′
4′	156.2	156			158.1		155.4		
5′	115.3	115.7	6.82 (2H, *d*, *J* = 8 Hz)	C-1′	113.3		115.6	6.80 (2H, *d*, *J* = 7.8 Hz)	C-3, C-4′
6′	130	130.3	7.32 (2H, *d*, *J* = 8 Hz)	C-1′, C-4′	128.3	7.08 (1H, *s*)	130.2	7.31 (2H, *d*, *J* = 7.8 Hz)	C-3, C-4′
1″		28.6	2.88 (1H, *dd*, J = 14.8, 8.4 Hz) 3.18 (1H, *bd*, *J* = 14.8 Hz)	C-2″, C-5, C-6, C-7	27.9	3.18 (2H, *d*, *J* = 5.6 Hz)			
2″	77.8	77.5	4.37 (1H, *bd*, J = 8.4 Hz)	C-6	122.6	5.20 (1H, *t*, *J* = 6 Hz)	91.2	4.78 (1H, *dd*, *J* = 9.3, 7.7 Hz)	
3″	115.1	146.8			132.4		27.1	3.14 (1H, *dd*, *J* = 7.7, 15.7 Hz) 3.22 (1H, *dd*, *J* = 9.3, 15.7 Hz)	C-7, C-8, C-9, C-2″, C-4″
4″	128.1	110.4	4.86 (1H, *s*) 4.99 (1H, *s*)	C-2″, C-5″	17.7	1.65 (3H, *s*)	72.3		
5″	27.9	18.5	1.85 (3H, *s*)	C-2″, C-3″, C-4″	25.7	1.69 (3H, *s*)	24	1.23 (3H, *s*)	C-2″, C-4″, C-6″
6″	27.9						25.6	1.36 (3H, *s*)	C-2″, C-4″, C-5″
1‴		21.9	3.47 (2H, *d*, *J* = 7.2 Hz)	C-2‴, C-3‴, C-7, C-8, C-9			22	3.38 (2H, *d*, *J* = 7.3 Hz)	C-5, C-6, C-7, C-2‴, C-3‴
2‴		122.2	5.22 (1H, *t*, *J* = 7.2 Hz)	C-1‴, C-3‴, C-7, C-8, C-9			121.5	5.21 (1H, *t*, *J* = 7.3 Hz)	
3‴		132.3	–	–			132.4		
4‴		17.9	1.81 (3H, *s*)	C-3‴, C-5‴			17.8	1.79 (3H, *s*)	C-2‴, C-3‴, C-5‴
5‴		25.8	1.69 (3H, *s*)	C-4‴			25.7	1.69 (3H, *s*)	C-2‴, C-3‴, C-4‴
OCH_3					55.4	3.72 (3H, *s*)			

[a] Assignments made by combination of COSY, DEPT and HMQC experiments.
[b] $CDCl_3/CD_3OD$.
[c] $CDCl_3$.

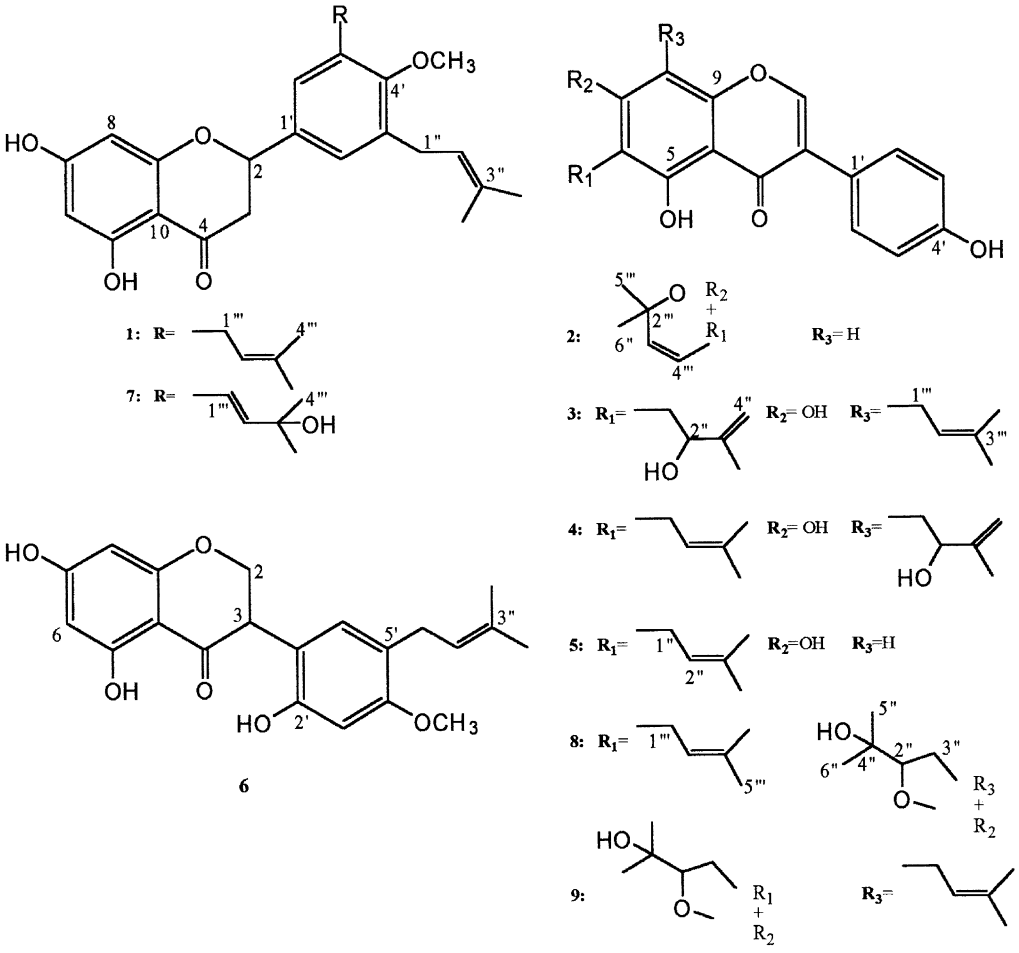
while signals at δ 2.88, 3.18, 4.37, 4.86, 4.99 and 1.85 with their corresponding carbons were assigned for a 2″ hydroxy prenyl group. Both physical and spectral data of **3** were different form those reported for erysenegalensein E (**4**) (Wandji et al., 1994a) suggesting an isomeric arrangement of the prenyl groups in **3**. A direct evidence for the exact positions of the prenyl and the hydroxy prenyl groups were derived from an HMBC (Table 1) experiment where the 2H-1‴ protons of the prenyl group at δ 3.47 showed three bonds correlation with both C-7 and C-9; 2 bonds correlation with C-8. The 2H-1″ protons of the hydroxy prenyl group at δ 2.88, 3.18 showed two bonds correlation with C-6, three bonds correlation with both C-5 and C-7. Finally the H-2″ proton of the hydroxy prenyl at δ 4.37 showed a three bonds correlation to C-6. These results allowed the unambiguous assigment for the hydroxy prenyl to position 6 and the prenyl to position 8 and **3** was identified as 5, 7, 4′-trihydroxy-8-(3‴-methylbut-2‴-enyl)-6-(2″-hydroxy-3″-methylbut-3″enyl) isoflavone and was given the name isoerysenegalensein E.

Compound **8** showed close similarities to compound **3** with two major differences. Its UV data (see Experimental) indicated that the OH at C-7 was not free since NaOAc failed to produce any bathochromic shift. ^{1}H and ^{13}C NMR data (Table 1) as well as the mass fragment at m/z 363 [$M^{+}-(CH_3)_2C{=}OH$] indicated that the hydroxy prenyl in **3** was replaced by a hydroxyisoprenyldihydrofuran moeity in **8** (Tahara et al., 1984). That moeity could form a ring structure including either C-6 or C-8 and C-7 OH. The spectral data of **8** were closely similar to those reported for senegalensin (**9**) (Wandji et al., 1990, 1994b; Nkengfack et al., 1991), however a major difference was observed in the UV spectrum with $AlCl_3$. In an HMBC experiment (Table 1) the exact position of C-9 at 154.9 ppm was unambiguously assigned due to its correlation to H-2 proton at δ 7.88. The correlations of the 2H-3″ at δ 3.14 and 3.22 with both C-7 and C-9 confirmed that the hydroxyisoprenyldihydrofuran formation utilized C-8 rather than C-6. Moreover, the 2H-1‴ protons of the prenyl at δ 3.38 showed correlation′s to both C-5 and C-7 indicating its C-6 location and **8** was consequently identified as 5,4′-dihydroxy-6-(3‴-methylbut-2‴-enyl)-2″-hydroxyisopropyl dihydrofurano [4″,5″:8,7] isoflavone and was given the name isosenegalensin.

The EI–MS (see Experimental) of compound **6** showed an M^{+} at m/z 370 consistent with the molecular formula $C_{21}H_{22}O_6$. All the carbons were clear in the ^{13}C NMR (Table 1) spectrum and were sorted by DEPT experiments into two CH_3, one OCH_3, two CH_2, six CH and 10 fully substituted carbons. The UV absorption bands at 211, 289 nm (see Experimental), 2H-2 at δ 4.61, 4.76, C-2 at 69.6 ppm, 1H-3 at δ 3.98 and C-3 at 45.3 ppm (Table 1 and Experimental) were all diagnostic for an isoflavanone skeleton (Mabry et al., 1970; Agrawal, 1989). In the UV spectrum the 20 and 36 nm bathochromic shift exhibited with $AlCl_3$ and NaOAc suggested the presence of free hydroxyl groups at positions 5 and 7 of ring A (Mabry et al., 1970). The two overlapped protons at δ 5.92 (these signals were resolved in the ^{1}H NMR measured in CD_3OD, see experimental) correlated to two carbons at 96.9 and 95.5 ppm were assigned for H-6 and H-8 respectively. This was further supported by the mass fragment at m/z 153 $[C_7H_5O_4]^{+}$ representing ring A with two hydroxyl groups. The mass fragment at m/z 218 $[C_{14}H_{18}O_2]^{+}$ representing ring B, confirmed the presence of a methoxyl, a hydroxyl and one (3″-methylbut-2″-enyl) groups. This fragmentation pattern is in full agreement with that reported for prenylated isoflavanones (Nkengfack et al., 1994). In the ^{1}H NMR the two aromatic singlets at δ 6.43 and 7.08 as well as the chemical shift of ring B carbons were in favour of a 2′, 4′, 5′ trisubstitution with the hydroxy and methoxy groups are *meta* oriented (Shirataki et al., 1982; Nkengfack et al., 1994). The exact positions of ring B substituents were unambiguously assigned by a GOESY experiment, where irradiation of the OCH_3 signal resulted in an enhancement at H-3′ and 2H-1″ of the prenyl; irradiation of the 2H-1″ of the prenyl enhanced both the OCH_3 and H-6′ signals. Compound **6** has a 0.0 $[\alpha]_D^{25}$ (MeOH; *c* 1.0) indicating a racemic mixture. The 3*R*, 3*S* configuration was assigned at C-3 since it is the only chairal center in the molecule. Based on the above discussion **6** was identified as 5,7,2′-trihydroxy-4′-methoxy-5′-(3″-methylbut-2″-enyl) isoflavanone and was given the name lysisteisoflavanone.

The known flavonoids abyssinone V-4′-methylether **(1)** (Yenesew et al., 1998), alpinumisoflavone **(2)** (Olivares et al., 1982), wighteone **(5)** (Ingham et al., 1977) and burttinone **(7)** (Yenesew et al., 1998) were identified by comparing their data with those in the literature. ^{13}C NMR data for compound **2** (Table 1) is reported for the first time.

Flavonoids **7** and **8** were selected by the NCI for evaluation as anticancer agents through the 60 cell panel (Monks et al., 1991). The IC_{50}'s for **7** were less than 50 μM against 43 cell lines. Compound **7** showed maximum cytotoxicity against colon cancer cell line HCC-2998 ($IC_{50} = 20$ μM), while the IC_{50} were higher than 50 μM in all the five tested leukemia cell lines. Compound **8** was much less cytotoxic than **7**. The IC_{50}'s were less than 50 μM only against the four cell lines: ovarian cancer IGROV1 (45 μM), non-small cell lung carcinoma NCI-H322M (46 μM), colon cancer HCC-2998 (48 μM) and renal cancer UO-31 (49 μM). IC_{50}'s were higher than 100 μM against nine cell lines. Both compounds were not selective in their action against any tested panel indicating that they are general cytotoxic agents of no clinical value. Consequently no further investigation was carried out.



3. Experimental

3.1. General

Melting points were determined using Kofler's hot stage instrument and are uncorrected. UV spectra were determined using a UV-1201 Shimadzu spectrometer, while CD curves were determined using a JASCO J 720 spectrophotometer. NMR spectra were recorded on a Varian Unity 400 NMR instrument at 399.951 MHz for ^{1}H and 100.578 MHz for ^{13}C. EI–MS were taken on a VG 7070 E-HF.

3.2. Plant material

The stem bark of *E. lysistemon* Hutch. was collected from Alexandria (Cultivation garden) during October 1998. The plant was identified by Professor Nabil El-Hadidi, Botany Department, Faculty of Science, University of Cairo. A voucher sample (EE2) is deposited in the Department of Pharmacognosy, Faculty of Pharmacy, University of Alexandria.

3.3. Extraction, isolation and characterization

Fresh and sliced stem bark (2.5 kg) of *E. lysistemon* was extracted with CH_2Cl_2 by percolation at room temperature. The CH_2Cl_2 extract was concentrated under reduced pressure at 35 °C to produce 14 g of dark brown residue. The crude extract was subjected to fractionation on silica gel column (400 g, 5 cm diameter) eluted with light petroleum then light petroleum–EtOAc mixtures.

Fractions eluted with 18% EtOAc in pet ether (100 mg) were further purified on PTLC using light petroleum/EtOAc (7:3) as eluting system. The major band (R_f value = 0.58) was eluted with CHCl3/MeOH (1:1) to give 14 mg of **1**.

Fractions eluted with 20% EtOAc in light petroleum (800 mg) were further purified by column chromatography (20 g, 2.5 cm diameter) eluting with light petroleum then light petroleum/EtOAc mixtures. Fraction 7, eluted with 18% EtOAc in light petroleum, afforded 61 mg of **2** as yellow needles (R_f value = 0.49, light petroleum/ether, 1:1) after recrystallization from light petroleum/EtOAc.

Fractions eluted with 25% EtOAc in light petroleum (190 mg) were further purified on siliga gel column followed by PTLC, using light petroleum/ether (1:1) as the eluting system, to afford 25 mg of **3** ($R_f = 0.46$).

Fractions eluted with 30% EtOAc in light petroleum (500 mg) were subjected to PTLC using CH_2Cl_2/MeOH (9:1) as the eluting system. The major three bands were collected and eluted with methanol. The less polar band ($R_f = 0.45$) component was identified as **5** (31 mg), whilst the more polar band ($R_f = 0.34$) component was identified as **6** (118 mg).

The most polar band ($R_f = 0.23$) (178 mg) was further separated on RP_{18} PTLC plates [MeOH/H_2O (8:2)] to give **7** ($R_f = 0.54$) as the less polar band and **8** ($R_f = 0.35$) as the most polar one.

3.3.1. Isoerysenegalensein E (lysisteisoflavone) (*3*)

Yellow crystals ($CHCl_3$), m.p. 155–156 °C λ_{max}^{MeOH} nm: 341 (sh), 272, 203, $\lambda_{max}^{MeOH+NaOMe}$ nm: 331 (sh), 284, 210, $\lambda_{max}^{MeOH+AlCl_3}$ nm: 360 (sh), 271, 213, $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm: 362 (sh), 275, 212, $\lambda_{max}^{MeOH+NaOAc}$ 341 (sh), 284, 215; ^1H and ^{13}C NMR Table 1; EIMS *m/z* (rel. int.) 422 $[M]^+$ (8), 351 $[M^+ - C_4H_7O]$ (33), 321 (26), 295 $[M^+ - (C_4H_7O + C_4H_8)]$ (100), 177 $[A^+ - (C_4H_7O + C_4H_8)]$ (9), 118 $[B^+ (C_8H_6O)]$ (8), 55 (8), 43 (23).

3.3.2. Lysisteisoflavanone (*6*)

Dark yellow amorphous solid, m.p. 98 °C; $[\alpha]_D^{25}$ 0.0 (MeOH; *c* 1.0) λ_{max}^{MeOH} nm: 326 (sh), 289, 230 (sh), 211, $\lambda_{max}^{MeOH+NaOMe}$ nm: 325, 214, $\lambda_{max}^{MeOH+AlCl_3}$ nm: 363 (sh), 309, 275 (sh), 217, $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm: 364 (sh), 308, 275 (sh), 217, $\lambda_{max}^{MeOH+NaOAc}$ 325, 287 (sh), 217; ^1H and ^{13}C NMR in $CDCl_3$ Table 1; ^1H NMR (400 MHz, CD_3OD): δ ; 1.59 (3H, *s*, H-4″), 1.65 (3H, *s*, H-5″), 3.12 (2H, *bd*, $J = 7.4$ Hz, H-1″), 3.30 (3H, *s*, O-CH_3), 4.13 (1H, *dd*, $J = 5$, 9.5 Hz, H-3), 4.38 (1H, *dd*, $J = 5$, 10.9 Hz, H-2), 4.52 (1H, *dd*, $J = 9.5$, 10.9 Hz, H-2), 5.20 (1H, *t*, $J = 7.4$ Hz, H-2″), 5.86 (1H, *d*, $J = 2$ Hz, H-6), 5.89 (1H, *d*, $J = 2$ Hz, H-8), 6.43 (1H, *s*, H-3′), 6.70 (1H, *s*, H-6′); EIMS *m/z* (rel. int.) 370 $[M]^+$ (100), 315 $[M^+ - C_4H_7]$ (5), 229 (21), 218 ($C_{14}H_{18}O_2$, 64), 203 ($C_{13}H_{15}O_2$, 71), 191 (17), 175 (18), 163 (12), 153 ($C_7H_5O_4$, 54), 69 (C_5H_9, 73), 55 (C_4H_7, 19), 43 (C_3H_7, 21).

3.3.3. Isosenegalensein (*8*)

Yellow crystals ($CHCl_3$), m.p. 158 °C; λ_{max}^{MeOH} nm: 271, 216, $\lambda_{max}^{MeOH+NaOMe}$ nm: 277, 216, $\lambda_{max}^{MeOH+AlCl_3}$ nm: 326 (sh), 275, 218 $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm: 364 (sh), 325 (sh), 283, 216, $\lambda_{max}^{MeOH+NaOAc}$ nm: 270, 219; ^1H and ^{13}C NMR Table 1; EIMS *m/z* (rel. int.) 422$[M]^+$ (51), 407 $[M^+ - CH_3]$ (14), 389 (16), 363 $[M^+ - (CH_3)_2C=OH]$ (17), 349 (26), 321 (12), 307 $[M^+ - ((CH_3)_2C=OH + C_4H_8)]$ (9), 295 (24), 118 $[B^+]$ (7), 69 (C_5H_9) (17), 59 $[(CH_3)_2C=OH]^+$ (100), 43 (C_3H_7) (56).

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